



Year: 2016

Surface roughness of dental implants and treatment time using six different implantoplasty procedures

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Abstract: OBJECTIVES: To test whether or not one of six implantoplasty procedures is superior to the others rendering a minimal final implant surface roughness and a short treatment time. MATERIAL AND METHODS: Forty-two one-piece implants were embedded in epoxy resin blocks with 6-mm rough implant surface exposed. The following implantoplasty polishing sequences were applied: Brownie® , Greenie® sequence (BG) (diamond rotary instruments 106-, 40-, 15-µm grit, Brownie® , Greenie® silicone polishers); Arkansas stone sequence (AS) (diamond 106-, 40-, 15-µm grit, Arkansas stone torpedo-shaped bur); Short diamond sequence (SD) (diamond 106-, 40-, 4-µm grit); Short diamond sequence with Greenie® (SDG) (diamond 106-, 40-, 4-µm grit, Greenie®); Complete diamond sequence (CD) (diamond 106-, 40-, 15-, 8-, 4-µm grit); Complete diamond sequence with Greenie® (CDG) (106-, 40-, 15-, 8-, 4-µm grit, Greenie®). The polished neck portion served as a positive control, the untreated sandblasted and acid-etched surface as negative control. Each implant was scanned with a contact profilometer rendering Ra values and Rz values as a measure of surface roughness. The time needed to polish the implant surface for each group was recorded. Simultaneous comparisons between more than two groups were done performing Kruskal-Wallis tests. Comparisons between two groups were analysed using Wilcoxon rank-sum tests. RESULTS: Mean Ra values amounted to $0.32 \pm 0.14 \mu\text{m}$ (BG), $0.39 \pm 0.13 \mu\text{m}$ (AS), $0.59 \pm 0.19 \mu\text{m}$ (SDG), $0.71 \pm 0.22 \mu\text{m}$ (SD), $0.75 \pm 0.26 \mu\text{m}$ (CDG), $0.98 \pm 0.30 \mu\text{m}$ (CD), $0.10 \pm 0.01 \mu\text{m}$ (PC) and $1.94 \pm 0.47 \mu\text{m}$ (NC). Pairwise one-sided comparisons between the test group revealed statistically significant differences ($P < 0.05$). The shortest treatment time was recorded for group AS ($13 \pm 2 \text{ min}$) and the longest for CDG ($21 \pm 2 \text{ min}$) and BG ($21 \pm 4 \text{ min}$). CONCLUSIONS: Considering final surface roughness and treatment duration, the use of rotary diamond burs in decreasing roughness, followed by an arkansas stone (group AS), appears to be an optimal treatment option.

DOI: <https://doi.org/10.1111/clr.12682>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-113944>

Journal Article

Accepted Version

Originally published at:

Ramel, Christian F; Lüssi, Anja; Özcan, Mutlu; Jung, Ronald E; Hämmerle, Christoph H F; Thoma, Daniel S (2016). Surface roughness of dental implants and treatment time using six different implantoplasty procedures. *Clinical Oral Implants Research*, 27(7):776-781.

DOI: <https://doi.org/10.1111/clr.12682>

Surface roughness of dental implants and treatment time using six different implantoplasty procedures

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Short title: Surface roughness of dental implants following implantoplasty

Key words: Dental implants, implantoplasty, implant surface modification, peri-implantitis, profilometry, resective peri-implantitis therapy, rotary instruments

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Abstract

Objectives: to test whether or not one out of six implantoplasty procedures is superior to the others rendering a minimal final implant surface roughness and a short treatment time. **Material and methods:** Forty-two one-piece implants were embedded in epoxy resin blocks with 6 mm rough implant surface exposed. The following implantoplasty polishing sequences were applied: Brownie[®], Greenie[®] sequence (BG) (diamond rotary instruments 106 µm, 40 µm, 15 µm grit, Brownie, Greenie silicone polishers); Arkansas stone sequence (AS) (diamond 106 µm, 40 µm, 15 µm, Arkansas stone torpedo shaped bur); Short diamond sequence (SD) (diamond 106 µm, 40 µm, 4 µm grit); Short diamond sequence with Greenie[®] (SDG) (diamond 106 µm, 40 µm, 4 µm grit, Greenie[®]) Complete diamond sequence (CD) (diamond 106 µm, 40 µm, 15 µm, 8 µm, 4 µm); Complete diamond sequence with Greenie[®] (CDG) (106 µm, 40 µm, 15 µm, 8 µm, 4 µm, Greenie[®]). The polished neck portion served as a positive control, the untreated sand-blasted and acid-etched surface as negative control. Each implant was scanned with a contact profilometer rendering R_a values as a measure of surface roughness. The time needed to polish the implant surface for each group was recorded. Simultaneous comparisons between more than two groups were done performing Kruskal-Wallis tests. Comparisons between two groups were analysed using Wilcoxon rank sum tests. **Results:** Mean R_a values amounted to 0.32 ± 0.14 µm (BG), 0.39 ± 0.13 µm (AS), 0.59 ± 0.19 µm (SDG), 0.71 ± 0.22 µm (SD), 0.75 ± 0.26 µm (CDG), 0.98 ± 0.30 µm (CD), 0.10 ± 0.01 µm (PC) and 1.94 ± 0.47 µm (NC). Pairwise one-sided comparisons between the test group revealed statistically significant differences ($p < 0.05$). The shortest treatment time was recorded for group AS (13 ± 2 min) and the longest for CDG (21 ± 2 min) and BG (21 ± 4 min).

Conclusions: Considering final surface roughness and treatment duration, the use of rotary diamond burs in decreasing roughness, followed by an arkansas stone (group AS),

appears to be an optimal treatment option.

Introduction

Peri-implantitis has been introduced initially as a term for infectious pathological conditions of peri-implant tissues (Levignac 1965; Mombelli et al. 1987). Later on, it was agreed that this term should be used specifically for destructive inflammatory processes around osseointegrated implants in function that lead to peri-implant pocket formation and progressive loss of supporting bone (Albrektsson & Isidor 1994). The prevalence of peri-implantitis is estimated to affect 10% of the implants and 20% of the patients up to 5 to 10 years following implant placement (Mombelli et al. 2012). Among many other factors, smoking and history of periodontitis are significantly associated with the development of peri-implantitis (Galindo-Moreno et al. 2005; Heitz-Mayfield 2008; Roccuzzo et al. 2010, 2012). Since peri-implantitis may lead to complete disintegration and implant loss, it is crucial to intervene and try to stop the progression of the disease or even attempt to achieve a “restitutio ad integrum” by regenerating the lost tissues.

To date, there are no standardized, generally accepted, evidence-based treatment protocols for the treatment of peri-implant infections (Renvert et al. 2012). Some case series and clinical trials, however, indicate a beneficial effect of resective (Romeo et al. 2005, 2007) or a combined resective and regenerative (Matarasso et al. 2014; Schwarz et al. 2011, 2014; Suh et al. 2003) surgical approach with a modification of the implant surface, the so-called “implantoplasty”.

The objective of a resective therapy is to reduce the severity of the inflammatory reaction and to re-establish a physiological biologic width by reducing pocket depth. In addition to soft tissue excision and osteotomy in an attempt to create a favourable bone architecture, implantoplasty may be indicated usually consisting of removing the implant threads and smoothening rough implant surfaces with rotary instruments. The purpose of

implantoplasty is firstly to polish the implant surface, thereby removing the entire outmost infected layer of titanium and creating a new sterile surface structure and secondly to render the affected implant surface less plaque-retentive by reducing the surface roughness.

In a clinical setting, most frequently diamond burs or carbide bone cutters (composed of tungsten and carbon alloy) are used to remove the threads of the exposed implant surface. This is then followed by the use of silicone polishers to smoothen the rough implant surface (Meier et al. 2012; Romeo et al. 2005, 2007; Schwarz et al. 2011, 2014). Some clinicians additionally use Arkansas burs after the diamond burs in order to further reduce roughness before using silicone polishers, as done in a recent clinical case series study (Matarasso et al. 2014). A number of clinical split-mouth studies reported a threshold value (R_a of 0.2 μm) to be adequate in terms of final surface roughness (Bollen et al. 1996; Quirynen et al. 1996). These studies indicated that polishing below this threshold value does not impact the total amount of plaque or the pathogenicity of the colonizing bacteria significantly (Bollen et al. 1996; Quirynen et al. 1996). Apart from the fact that implantoplasty is a very time consuming process, debris from the implant (i.e. contaminated titanium particles) as well as particles originating from rotating instruments can be dispersed into the surrounding hard and soft tissues. Carbide and diamond coatings on bur surfaces are harder than titanium and do not wear off extensively. Silicone polishers, however, produce large amounts of silicone particles that may eventually cause immunological reactions and interfere with the healing process following this type of therapy. This is the reason some authors abstain from using abrasive silicone polishers and use Arkansas burs instead (Schwarz et al. 2011, 2014). The goal would therefore be to optimize the polishing process, minimize the formation of debris and reduce the treatment time.

The objectives of this study were therefore to test whether or not one out of six implantoplasty procedures is superior rendering a minimal final implant surface roughness and a short treatment time.

Materials and methods

Implants

Forty-two implants (Standard Plus, Regular Neck, SLA®, 4.8 Ø mm, length 10 mm, Institut Straumann AG, Basel, Switzerland) were embedded in epoxy resin moulds (1.5 cm x 1.5 cm x 1.5 cm) in such a way that 6 mm of rough surface was exposed, resembling a horizontal peri-implant defect with only supracrestal aspects. These implants were subjected to 6 different protocols of implantoplasty using rotating burs and polishers. While the industrially polished neck portion served as a positive control, the untreated double sand-blasted and acid-etched (SLA) surface served as a negative control.

Burs

The following burs were used under copious irrigation with water: a) at 200'000 rpm bud shaped diamond rotary instruments, short neck: 106 µm, 40 µm and 15 µm grit in sequence (Intensiv SA, Montagnola, Switzerland); b) at 40'000 rpm experimental flame shaped diamond rotary instruments, long neck: 8 µm and 4 µm grit (Intensiv SA, Montagnola, Switzerland); c) at 20'000 rpm Arkansas stone torpedo shaped white aluminium oxide bur (Jota AG, Rüti, Switzerland); d) at 20'000 rpm mini-point shaped abrasive impregnated silicone polishers (Brownie®, Greenie®, Shofu Dental GmbH, Ratingen, Germany).

Implantoplasty procedures

The following 6 procedures served as test groups:

1. Group BG = Brownie[®], Greenie[®] sequence

(Diamond burs 106 µm, 40 µm, and 15 µm grit, Brownie[®], Greenie[®])

2. Group AS = Arkansas stone sequence

(Diamond burs 106 µm, 40 µm, and 15 µm grit, Arkansas stone)

3. Group SD = Short diamond sequence

(Diamond burs 106 µm, 40 µm and 4 µm grit)

4. Group SDG = Short diamond sequence with Greenie[®]

(Diamond burs 106 µm, 40 µm and 4 µm grit, Greenie[®])

5. Group CD = Complete diamond sequence

(Diamond burs 106 µm, 40 µm, 15 µm, 8 µm, and 4 µm grit)

6. Group CDG = Complete diamond sequence with Greenie[®]

(Diamond burs 106 µm, 40 µm, 15 µm, 8 µm, and 4 µm grit, Greenie[®])

The industrially polished implant neck served as a positive control (PC) and the rough SLA implant surface (endosseous part) as a negative control (NC).

One calibrated individual (AL) performed all implantoplasty procedures (6 implants per group) under standardized conditions. A hand-held contra-angle hand-piece (at 200.000 rotations per minute/rpm) under irrigation with water was used for diamond burs with a grit size of ≥ 15 µm. For the remaining instruments, a contra-angle hand-piece working with maximal 40.000 rpm was used, again with copious irrigation. In all sequences, the burs were used with decreasing grit sizes, starting with the bur having the largest grit size. With this bur, the exposed implant surface was polished until it had an evenly machined appearance as determined by the naked eye. This procedure was continued to the finest instrument (smoothest grit size). For each implant a new set of instruments was used. The duration of the entire polishing sequence per implant was recorded for each group in

minutes.

(Figure 1a-d.)

Surface roughness measurements

Each implant was scanned with a stylus profilometer (Mahr Perthometer S2, Mahr, Göttingen, Germany). By the use of a diamond tip, the surface roughness was measured along a straight line at a constant speed and a constant pressure. The epoxy mould retaining the implant was fixed in a way that the profilometer tip was rectangular to the implant surface and the needle moved along the implant axis. The profilometer scanned along a length of 2.143 mm using the inner third of this distance. The following roughness parameters were then calculated:

R_a (arithmetic mean roughness): R_a is the mean of the absolute values of the modified roughness profile, based on the central line to a reference route.

R_z (averaged roughness): R_z is the arithmetic mean of the differences between the five highest and five lowest points of a profile within a sample route on the surface measured.

The vertical movements of the tip, which are triggered by the surface irregularities, were transferred to a transducer. This transducer generated an electrical stimulus, which was digitized and recorded. Five measurements were performed in parallel direction on each of the four axial sides of the implant yielding 20 measurements per implant. Outer equidistant margins from both ends of each specimen (0.714 mm per site) were not considered for the calculation.

Statistical analysis

All statistical analyses were calculated with R 3.1.0.

Non-parametrical methods were applied due to non-normally distributed data. The absence of normality was checked computing QQ plots as well as p values according to Shapiro Wilk's test ($p < 0.0001^*$ for both R_a , R_z).

Simultaneous comparisons between more than two groups were done performing Kruskal-Wallis tests. Comparisons between two groups were analysed using Wilcoxon rank sum tests. The latter were corrected for multiple testing (Holm's method).

Level of significance was set to 0.05.

Results

The data were non-normally distributed for the independent variables of " R_a ", " R_z ". All descriptive data are presented in Table 1, whereas Table 2a-b displays the statistical analyses with p-values.

(Table 1.)

Surface roughness

(Figure 2.)

In ascending order, mean R_a values of test groups amounted to $0.32 \pm 0.14 \mu\text{m}$ (BG), $0.39 \pm 0.13 \mu\text{m}$ (AS), $0.59 \pm 0.19 \mu\text{m}$ (SDG), $0.71 \pm 0.22 \mu\text{m}$ (SD), $0.75 \pm 0.26 \mu\text{m}$ (CDG), $0.98 \pm 0.30 \mu\text{m}$ (CD). Mean R_a values of control groups were $0.10 \pm 0.01 \mu\text{m}$ (PC) and $1.94 \pm 0.47 \mu\text{m}$ (NC). There are significant differences between some of the test and control groups ($p < 0.0001^*$). Pairwise one-sided comparisons between every two of the eight groups are displayed in Table 2a. BG is significantly superior (alternative hypothesis: superiority, i.e. lower value) to every other test group and to NC. (Table 2a.).

Mean R_z values of test groups were as follows: $2.31 \pm 0.95 \mu\text{m}$ (BG), $3.19 \pm 1.17 \mu\text{m}$ (AS), $4.35 \pm 1.37 \mu\text{m}$ (SDG), $5.21 \pm 1.77 \mu\text{m}$ (CDG), $5.39 \pm 1.84 \mu\text{m}$ (SD), $6.86 \pm 2.20 \mu\text{m}$ (CD) and

mean R_z values of control groups amounted to $0.81 \pm 0.19 \mu\text{m}$ (PC) and $13.15 \pm 3.09 \mu\text{m}$ (NC). Pairwise one-sided comparisons between every two of the eight groups are displayed in Table 2b. BG is significantly superior (alternative hypothesis: superiority, i.e. lower value) to every other test group and to NC. (Table 2b.).

Treatment time

The least time-consuming bur sequence was group AS (13 ± 2 min) followed by group SD (12 ± 1 min) and group SDG (12 ± 2 min) (Table 2c.). The most time-consuming sequences were CDG (21 ± 2 min) and BG (21 ± 4 min), approximately requiring 70 % more time.

Discussion

The results of this study revealed that i) all procedures resulted in a reduction of the surface roughness of the original SLA surface, ii) the final implant surface roughness expressed as R_a values, R_z values and the time span necessary to perform the different treatment protocols varied extensively between the groups.

The roughness and the free surface energy of implant surfaces exposed to the oral environment may strongly influence the colonization of bacteria organized in biofilms (Teughels et al. 2006) and could as a consequence favour the development of peri-implant disease (Dohan Ehrenfest et al. 2011). In cases when both surface characteristics interact with each other, surface roughness was found to be the predominant factor (Teughels et al. 2006). The R_a value is the most common indicator to characterize surface roughness. It is, however, well known that R_a does not describe surface topography in great detail. It captures surface topography simply in one direction and is only as accurate as the diamond tip of the profilometer that is used for sensing the irregularities of the surface (Wennerberg & Albrektsson 2000). In the present study, the implants tested enabled accurate roughness evaluation since they have a cylindrical implant neck area without threads that could also serve as a positive control group.

A number of clinical split-mouth studies reported a threshold value (R_a of 0.2 μm) to be adequate in terms of final surface roughness (Bollen et al. 1996; Quirynen et al. 1996). These studies indicated that polishing below this threshold value does not impact the total amount of plaque or the pathogenicity of the colonizing bacteria significantly (Bollen et al. 1996; Quirynen et al. 1996). In addition, a recent study compared different rotary instruments for their effectiveness and efficiency to smoothen dental implant surfaces (Meier et al. 2012). In that study, one-piece implants were machined with carbide cutters

and a diamond bur. All obtained R_a values were higher than $0.5\ \mu\text{m}$. It was therefore postulated that, in addition, silicone polishers such as Brownies[®] and Greenies[®] should be used to further reduce the surface roughness. Several clinical studies applied implantoplasty procedures using carbide or diamond and Arkansas burs, followed by silicone polishers (Matarasso et al. 2014; Meier et al. 2012; Romeo et al. 2005, 2007; Schwarz et al. 2011, 2014). However, in none of the studies the final surface roughness was measured after the use of the silicone polishers.

The initial treatment with burs that are harder than titanium (i.e. carbide and diamond burs) aims to remove the intoxicated implant surface and the implant threads. One of the disadvantages associated with the use of such burs is the pollution of the surgical field caused by titanium particles. The same applies for the use of silicone polishers such as Brownies[®] and Greenies[®]. In addition, these instruments are being worn off themselves resulting in additional silicone debris potentially polluting the peri-implant wound bed.

The present study was designed to avoid the use of silicone polishers, thereby minimizing the debris pollution resulting from the instruments. For this purpose, superfine experimental diamond burs ($8\ \mu\text{m}$ and $4\ \mu\text{m}$) and an Arkansas stone topped shaped aluminum oxide bur were used instead of silicone polishers. The use of these superfine diamond burs resulted in a mean R_a surface roughness of $0.983\ \mu\text{m}$. The substitution of the silicone polishers with an Arkansas bur resulted in a mean R_a value of $0.394\ \mu\text{m}$. Both R_a values were significantly rougher compared to the one obtained using both silicone polishers (R_a : $0.318\ \mu\text{m}$). Using only a Greenie after the superfine diamonds did not enhance the values achieved by superfine diamonds greatly. The present study showed that the use of Brownies[®] and Greenies[®] is necessary in order to get the smoothest possible surface under clinic-like conditions. On the other hand, a clinician has to consider the pollution of the surgical site with silicone debris while polishing an implant surface with

Brownies[®] and Greenies[®]. Considering the fact that the replacement of the silicone polishers by an Arkansas bur left an only slightly rougher surface of R_a : 0.394 compared to R_a : 0.318, one might argue that from a clinical point of view it is advantageous to apply the AS procedure. In order to further elaborate on this clinical dilemma, studies are needed investigating the biotoxicity of the different kinds of debris being generated during an implantoplasty procedure and eventually a potential detrimental effect on clinical outcomes.

In the present study an effective smoothing of the SLA surface was achieved by using silicone polishers, Arkansas stones or superfine diamond burs. The question still remains, whether or not implantoplasty of the supracrestal infected implant surface can clinically result in a final surface roughness close to the postulated R_a threshold value (less than 0.2 μm) (Bollen et al. 1996; Quirynen et al. 1996). None of the methods applied in the present study was able to achieve these values in a setting close to a clinical situation. One might speculate that the suggested threshold in terms of surface roughness is only applicable under standardized industrial conditions. This is supported by the fact that the prefabricated polished neck portion only had a mean R_a of 0.103 ± 0.011 .

In a clinical environment, the goal of implantoplasty procedures is to remove the outmost titanium layers of the implant. This ideally results in a smooth and sterile surface. Given such an ideal surface structure, no further disinfecting methods would be needed for the supracrestal portion of the exposed implant threads. In addition, the removal of the implant threads produces an implant topography that is better accessible for patients and facilitates oral hygiene. Several studies demonstrated a beneficial effect on plaque formation when surfaces were reduced in roughness (Teughels et al. 2006). In a clinical study, a more mature plaque layer was found supragingivally on roughened (R_a : 0.8 μm) abutments after three months compared to standard abutments (R_a : 0.3 μm).

Submucosally, the rough surfaces harboured 25-fold more bacteria (Quirynen et al. 1993). In another more recent preclinical study, the spontaneous progression of ligature-induced peri-implantitis was examined (Berglundh et al. 2007) on standard SLA (S_a : 2.29 μm) and turned (S_a : 0.35 μm) implants placed in a dog model. After healing and osseointegration, ligatures were placed for 4 months in order to produce a peri-implantitis resulting in 40% bone loss. The ligatures were removed and the behaviour of the bone was studied for an additional 5 months. Around the turned implants no further breakdown was detected, whereas the standard implants experienced further progression of bone loss. Furthermore, histologically, both bone loss and the size of the connective tissue inflammatory lesion were more pronounced in SLA than in turned implant sites (Berglundh et al. 2007). This demonstrated the beneficial effect of smoother (turned) compared to rough implant surfaces and in part supports the use of an implantoplasty procedure.

Bone loss caused by peri-implantitis and its therapy is a complex clinical challenge. One limitation of this study is the fact that it only focuses on the treatment of the supracrestal aspect of the lesion. Often an infrabony area of a defect is also observed, which is contained by surrounding bone and is therefore suited for GBR procedures in order to augment the bone loss caused by the infection (Matarasso et al. 2014; Schwarz et al. 2011, 2014). Furthermore, the reaction concerning adhesion and colonization of bacterial plaque after implantoplasty procedures remains unclear.

One additional aspect which remains un-investigated yet, is the mechanical stability of the polished implant portion: Bone loss causes higher mechanical stress on the part of the implant which is located above the bone crest. Following implantoplasty procedures that result in a reduced implant diameter, an additional stress is potentially caused on the remaining titanium implant body. Further research should be directed to investigate the

surface biocompatibility by means of cell cultures and to test the implant stability following implantoplasty procedures.

Another factor of clinical importance is the time needed to perform a given treatment. In the present study, treatment duration depended on the treatment protocol and varied between 12 and 21 minutes with the groups SDG, AS and SD representing the fastest options. It is known that clinicians tend to shorten implantoplasty procedures (Sharon et al. 2013). In order to recommend a clinical procedure, an optimal balance between final surface roughness and overall treatment time needs to be found.

Conclusions

In conclusion, considering the treatment duration, production of debris and final surface roughness, the group beginning with the diamond bur of 106 μm , followed by 40 μm and 15 μm grit size and ending up with the Arkansas stone torpedo shaped aluminum oxide bur replacing the silicone polishers, appears to be the most optimal solution. Further studies are needed to evaluate these implantoplasty procedures concerning biocompatibility, biotoxicity of debris generated by implantoplasty procedures, implant fracture strength and in clinical settings assessing final surface roughness, time and long-term clinical stability of implants affected by peri-implantitis.

Acknowledgements and source of funding

The authors would like to acknowledge Mr. Heinz Frei, ITI, Basel, Switzerland for providing the implants, Intensiv GmbH, Montagnola, Switzerland, for providing the polishing instruments and Mr. Klaus Becker for his assistance with the roughness measurements.

Conflict of Interests

The authors declare that they have no conflict of interests related to this study.

Figure legends:

Table 1. R_a and R_z values stated in μm , duration of implantoplasty stated in minutes.

Table 2a. P values of pairwise one-sided comparisons (alternative hypothesis: superiority, i.e. lower value) considering R_a values. Groups differing statistically significantly ($p < 0.05$) are marked with an asterisk.

Table 2b. P values of pairwise one-sided comparisons (alternative hypothesis: superiority, i.e. lower value) considering R_z values. Groups differing statistically significantly ($p < 0.05$) are marked with an asterisk.

Table 2c. P values of two-sided Wilcoxon rank sum tests considering duration of implantoplasty. Groups differing statistically significantly ($p < 0.05$) are marked with an asterisk. Not statistically significant differences ($p > 0.05$) were recorded between BG and CDG, AS and SDG, AS and SD as well as SDG and SD. The corresponding p values are written in bold letters.

Figs. 1a-d. Embedded implants in epoxy resin (a) before polishing process (Baseline), followed by (b) AS, (c) BG and (d) CD.

Fig. 2. Mean R_a and R_z surface roughness values (μm) of the implant surfaces with respect to the different treatment protocols (in ascending order). All test groups resulted in rougher surfaces than the positive control and smoother surfaces than the negative control. Levels of significance of pairwise comparisons are displayed in table 2a (for R_a) and 2b (for R_z).

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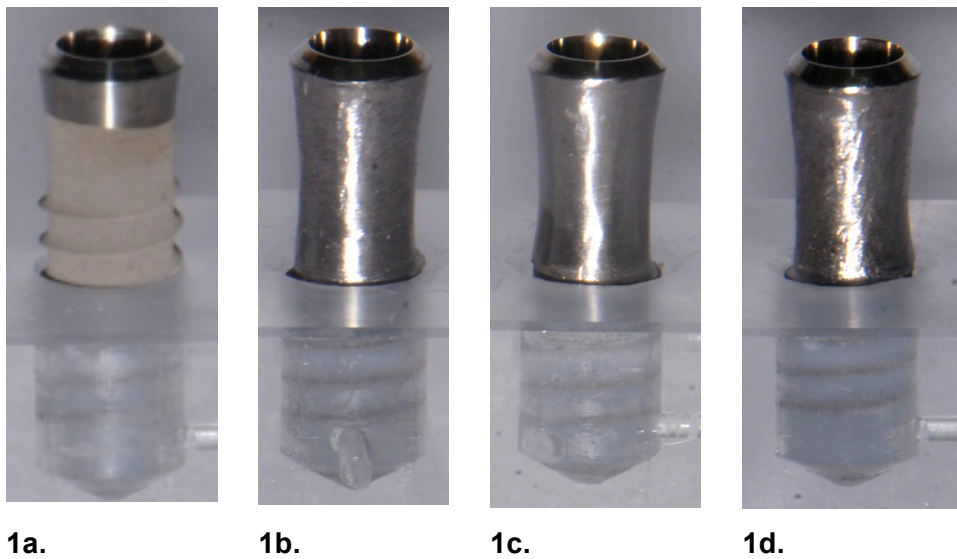
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Figures:



Figs. 1a-d.

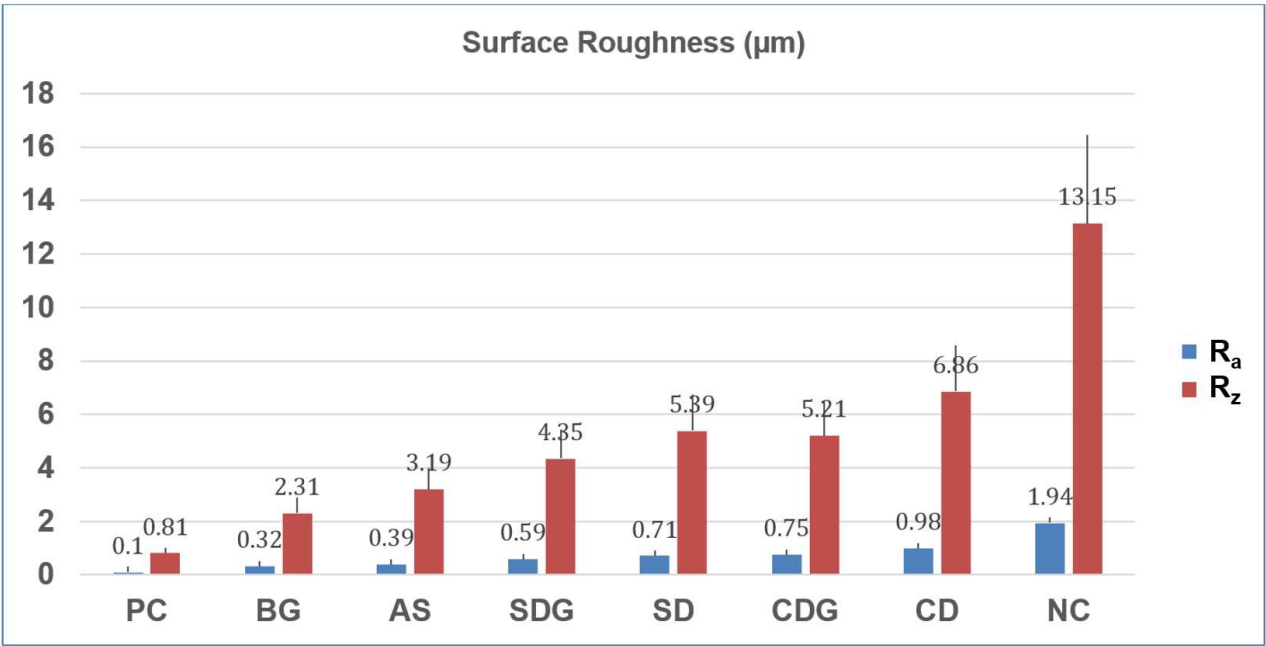


Fig. 2.

Tables:

	Group	R _a (Mean±SD)	Median	R _z (Mean±SD)	Median	Expenditure of time(Mean±SD)
1	PC	0.10±0.01	0.10	0.81±0.19	0.77	-
2	BG	0.32±0.14	0.28	2.31±0.95	2.10	21±4
3	AS	0.39±0.13	0.38	3.19±1.17	3.01	13±2
4	SDG	0.59±0.19	0.57	4.35±1.37	4.17	12±2
5	SD	0.71±0.22	0.69	5.39±1.84	5.06	12±1
6	CDG	0.75±0.26	0.72	5.21±1.77	5.10	21±2
7	CD	0.98±0.30	0.95	6.86±2.20	6.61	16±2
8	NC	1.94±0.47	1.89	13.15±3.09	12.90	-

Table 1.

	PC	BG	AS	SDG	SD	CDG	CD	NC
PC vs.	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
BG vs.	1.0000	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
AS vs.	1.0000	1.0000	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
SDG vs.	1.0000	1.0000	1.0000	-	<0.0005*	<0.0001*	<0.0001*	<0.0001*
SD vs.	1.0000	1.0000	1.0000	1.0000	-	1.0000	<0.0001*	<0.0001*
CDG vs.	1.0000	1.0000	1.0000	1.0000	1.0000	-	<0.0001*	<0.0001*
CD vs.	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	<0.0001*
NC vs.	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-

Table 2a.

	PC	BG	AS	SDG	SD	CDG	CD	NC
PC vs.	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
BG vs.	1.0000	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
AS vs.	1.0000	1.0000	-	<0.0001*	<0.0001*	<0.0001*	<0.0001*	<0.0001*
SDG vs.	1.0000	1.0000	1.0000	-	0.0002*	0.0015*	<0.0001*	<0.0001*
SD vs.	1.0000	1.0000	1.0000	1.0000	-	1.0000	<0.0001*	<0.0001*
CDG vs.	1.0000	1.0000	1.0000	1.0000	1.0000	-	<0.0001*	<0.0001*
CD vs.	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-	<0.0001*
NC vs.	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	-

Table 2b.